THERAPEUTIC HPV16 VACCINES

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application is a continuation of U.S. patent application Ser. No. 16/058,411, filed Aug. 8, 2018, which is a continuation of U.S. patent application Ser. No. 15/601,278 filed May 22, 2017, issued as U.S. Pat. No. 10,071,151 on Sep. 11, 2018, which is a continuation of U.S. patent application Ser. No. 14/932,789, filed Nov. 4, 2015, issued as U.S. Pat. No. 9,701,721 on Jul. 11, 2017, which claims priority under the Paris Convention from European Patent Application Serial No. EP 14 191 660.1, filed Nov. 4, 2014, the entire contents of the aforementioned applications are incorporated herein by reference.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0002] This application contains a sequence listing, which is submitted electronically via EFS-Web as an ASCII formatted sequence listing with a file name "688097_278U3_sequence_listing" and a creation date of Jan. 21, 2020, and having a size of 31.2 KB. The sequence listing submitted via EFS-Web is part of the specification and is herein incorporated by reference in its entirety.

TECHNICAL FIELD

[0003] The disclosure relates to the field of biotechnology and medicine and, more in particular, to nucleic acid constructs and polypeptides that can be used in therapeutic vaccines against human papillomavirus type 16.

BACKGROUND

[0004] The family of human papillomaviruses (HPVs) include more than 100 types (also referred to as subtypes) that are capable of infecting keratinocytes of the skin or mucosal membranes. Over 40 types of HPV are typically transmitted through sexual contact and HPV infections of the anogenital region are very common in both men and women. Some sexually transmitted HPV types may cause genital warts. Persistent infections with "high-risk" HPV types (e.g., types 16, 18, 31, 45)—different from the ones that cause skin warts—may progress to precancerous lesions and invasive cancer, e.g., of the cervix, vulva, vagina, penis, oropharynx, and anus. The majority of HPV infections are spontaneously cleared within one to two years after infection. In healthy individuals circulating Th1- and Th2-type CD4+ T-cells specific for the viral early proteins E2, E6 and E7 of HPV-16 as well as E6-specific CD8+ T-cells, migrate into the skin upon antigenic challenge, indicating that successful defense against HPV-16 infection is commonly associated with a systemic effector T-cell response against these viral early antigens. In a minority (~1%) of infected individuals, HPV infection persists, ultimately resulting in genital neoplastic lesions. Among the high-risk HPVs, HPV16 and HPV18 are the main cause of cervical cancer, together causing about 70% of the cases, and these two types also play a major role in other HPV-induced cancers such as anal and oropharyngeal cancer. Worldwide, HPV is one of the most important infectious agents causing cancer.

[0005] Vaccination against HPV is deemed a feasible strategy to reduce the incidence or effects of infection by HPV (van der Burg and Melief, 2011, *Curr. Opinion Immunol.* 23:252-257).

[0006] Prophylactic HPV vaccines based on virus-like particles (VLPs) formed by the (envelope) protein L1 of the HPV types 16 and 18, are very efficient in the prevention of persistent infection and the associated disease by HPV16 and HPV18. These vaccines are believed to provide sterile immunity via the induction of neutralizing antibodies against the L1 proteins. Addition of L1-based VLPs from additional high-risk HPV types may further increase the breadth of protection conferred by such vaccines.

[0007] However, while such vaccines can prevent initial infection (i.e., they result in prophylaxis), there is no evidence of a beneficial effect on established genital lesions caused by HPV16 and HPV18, so they are not considered therapeutic vaccines against HPV (Hildesheim et al., 2007, *JAMA* 298:743-53).

[0008] Despite the introduction of these prophylactic vaccines, large numbers of people have already had or are still at risk of having persistent high-risk HPV infections and, therefore, are still at risk of getting cancer. Therapeutic vaccines for the eradication of established HPV infections and associated diseases are an urgent unmet medical need.

[0009] Some attempts to address this need have been described. For example, clinical trials have been carried out with a variety of different vaccination strategies, such as a fusion protein consisting of a heat shock protein (Hsp) from *Mycobacterium bovis* and HPV-16 E7 or consisting of a fusion protein of E6, E7 and L2 from HPV-16 and HPV-18, chimeric L1-E7 VLPs, recombinant vaccinia viruses expressing either E6 and E7 of HPV-16 and HPV-18 or bovine papilloma virus E2, DNA vaccines expressing CTL epitopes of E6 and E7 of HPV-16 and HPV-18, a live-attenuated Listeria monocytogenes (Lm) that secretes the HPV-16 E7 antigen, and synthetic long-peptides (SLPs) comprising HPV-16 E6 and E7 peptides. While some of these approaches show some, but limited, clinical efficacy, most have failed, demonstrating that improvement of the current strategies is needed.

[0010] Integration of the early HPV proteins E6 and E7 is a necessary step in the process from infection to cancer and continuous expression of E6 and E7 is required for the maintenance of the neoplastic phenotype of cervical cancer cells. E6 and E7 are, therefore, considered good targets for therapeutic vaccination. As mentioned, some studies have shown that therapeutic vaccination of women infected with high-risk HPV can induce regression of existing lesions. Kenter et al. showed a durable and complete regression in 47% of patients having Vulvar Intraepithelial Neoplasia (VIN) using SLPs derived from the HPV16 E6 and E7 proteins and an adjuvant as a therapeutic vaccine (Kenter et al., 2009, *N. Engl. J. Med.* 361:1838-47). Similarly, a study in which a protein-based vaccine (TA-CIN, consisting of a fusion protein of HPV16 E6, E7 and L2) was combined with local immune modulation in VIN 2/3 patients, showed complete regression in 63% of patients (Daayana et al., 2010, *Br. J. Cancer* 102:1129-36). Possible drawbacks of the synthetic long peptides as a vaccine include manufacturability at large scale and costs associated therewith, the need for potentially reactogenic adjuvant and the associated adverse effects associated with immunization (especially pain and swelling). Due to the high level of discomfort it is not likely